REMARKS

I. Support for the Amendments

Claims 1 and 10-12 have been amended. Support for claims 1 and 10-12, as amended, can be found throughout the original specification as filed. More particularly, support for amended claims 1 and 10 can be found on p. 4, ll. 5-17; from p. 4, l. 28, to p. 5, l. 3; from p. 6, l. 29, to p. 7, l. 9; on p. 10, ll. 1-19; and in the Examples. Additional support for amended claim 10 can be found on p. 5, ll. 18-23; and on p. 10, ll. 21-30. Support for amended claims 11-12 can be found from p. 4, l. 19, to p. 5, l. 3; from p. 7, l. 30, to p. 8, l. 5; and in the Examples.

II. Status of the Claims

Claims 1-17 were originally filed with the application and were subject to a restriction requirement. Claims 1, 6-10, and 15-17 were stated by the Examiner to be generic. In the Response to the Election/Restriction Requirement, Applicants elected Group II species vascular endothelial growth factor (VEGF). The Examiner requested Applicants to identify claims readable on this species. Applicants noted that "[t]hese generic claims and claims dependent thereon are readable on Group II" species. Claims 4, 5, 13, and 14 have been withdrawn without prejudice to the filing of one or more divisional applications. As the Examiner notes, claims 1-3, 6-12, and 15-17 are currently under examination, with claims 1 and 10 being the independent claims. Claims 2, 3, and 6-9 are dependent on claim 1 or on claims dependent on claim 1, while claims 11, 12, and 15-17 are dependent on claim 10 or on claims dependent on claim 10.

III. Proposed Drawing Correction in Response to the Examiner's Objection to the Drawings

The Examiner has objected to the drawings, specifically to Figure 1. The Examiner states:

> Figure 1 comprises 1A-1C. The drawings are objected to because the drawing descrption only describes Figure 1 but fails to acknowledge each individual drawing. A proposed drawing correction or corrected drawings are required in reply to the Office Action to avoid abandonment of the application. The objection to the drawings will not be held in abeyance. (P. 2.)

Figures 1A-1C were originally on a single page, entitled "Figure 1." Applicants have proposed correcting Figures 1A-1C by the addition of a Figure 1 legend as shown in the attached Replacement Figures. In the event that the Replacement Figures do not meet with the approval of the Examiner or of the Draftsperson, Applicants would be willing to amend the description of the drawings in the specification to replace [[Figure 1]] with --Figures 1A-1C--.

IV. Rejection Under 35 U.S.C. §112, Second Paragraph, is Accommodated

The Examiner has rejected claims 1-3, 6-12, and 15-17 under 35 U.S.C. §112, second paragraph, "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." More particularly, the Examiner alleges:

> Claims 1-3, 6-12 and 15-17 are rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. The omitted steps are: how to determine the ability of the plasmid encoding an endothelial cell mitogen to produce a biologically active endothelial mitogen protein or how to determine the ability of the first or second plasmid encoding an endothelial cell mitogen to produce a biologically active endothelial mitogen protein. In other words, the method steps must refer back to the preamble.

Applicants hereby amend claims 1 and 10 in accordance with the Examiner's remarks and respectfully submit that the amendments to claims 1 and 10 overcome the Examiner's rejection. (Claims 2, 3, 6-9, 11, 12, and 15-17 are dependent on either claim 1 or claim 10.)

The Examiner further alleges:

> Regarding claims 10-12 and 15-17, the recitation of "with plasmid containing a gene encoding for an endothelial cell mitogen" on line 12 of claim 10 renders the claims indefinite because it is unclear which plasmid it is referring to. In other words, is it the first plasmid construct or the second? Similarly, the recitation of the term "the plasmid" in claim 11 and 12 also renders the claims indefinite, because it is unclear which plasmid it is referring to. (P. 3.)

Applicants hereby amend claim 10-12 in accordance with the Examiner's remarks. (Claims 15-17 are dependent on claim 10.)

Overall, Applicants respectfully submit that the amendments to claims 1 and 10-12 overcome the Examiner's rejection of claims 1-3, 6-12, and 15-17 under 35 U.S.C. §112, second paragraph. Moreover, Applicants respectfully submit that the present claims 1-3, 6-12, and 15-17 fulfill the requirements of 35 U.S.C.§112, second paragraph, and request the Examiner's reconsideration of these claims accordingly.

V. Rejection of Claims 1-3, 7-12, 16, and 17 under 35 U.S.C. §103(a) over Sugihara, in view of Buttke, is Traversed

The Examiner has rejected claims 1-3, 7-12, 16, and 17 under 35 U.S.C. §103(a) "as being unpatentable over Sugihara et al., in view of Buttke et al (Journal of Immunological Methods, 1993, Vol 157, p.233-240)." We disagree.

The Examiner alleges:

> Sugihara et al. teach a method for testing the biological activity of VEGF 115 and VEGF164 comprising a) **transiently transfecting NIH 3T3 cells** with a vector encoding VEGF; b) incubating HUVEC cells with conditioned media from the NIH 3T3 cells transfected with VEGFs or control vector; c) **determining the cell survival by thymidine incorporation** (see page 3037,2nd col., 3rd paragraph). Sugihara et al. further teach that **the level of cell survival** of HUVEC cells treated with conditioned media from either VEGF115 or VEGF164 is significantly higher (more than 25% fold) than that of the control cells (see Figure 5B). However, Sugihara et al. **do not teach measuring cell survival by tetrazolium (MTS)/formazan assay**.
>
> Buttke et al. teach a method of measuring cell survival by using an MTS/formazan assay. Buttke et al. teach that MTS can be reduced by living cells to yield a formazan product that can be assayed colorimetrically (see abstract). Buttke et al. further indicate that this method has the advantage of producing soluble formazan that eliminates the need of using detergent or organic solvent for extraction, rapid color development and storage stability (see abstract).
>
> It would have been obvious to one of ordinary skill of art to develop a method of testing the biological activity of a endothelial cell mitogen protein such as VEGF by measuring the survival rate of endothelial cells incubating with conditioned media collected from host cells transfected with vector encoding a endothelial cell mitogen based on the teaching of Sugihara et al. Methods of measuring cell survival are well known in the art. Such method includes MTS/formazan assay taught by Buttke et al. One of ordinary skill in the art would have been motivated to use MTS/formazan assay because its advantage over other assay including rapid color development, storage stability and elimination of the use of detergent or organic solvent extraction step. The level of skill in the art is high. Absent evidence from the contrary, one of ordinary skill in the art would have reasonable expectation of success to practice the method as claimed. Therefore, the invention is *prima facie* obvious to one of ordinary skill of art at the time the invention was made. (Pp. 4-5; italics in original; bold emphasis added.)

Applicants respectfully disagree with the Examiner's comments and traverse the obviousness rejection.

First, with respect to the Examiner's point a) regarding Sugihara, Applicants respectfully submit that the transfection assay method described therein would appear to involve **stable, rather than transient, transfection**. In the description of the Transfection Assay and Assay for Mitogenic Activity (p. 3034), the transfection took place in an NIH 3T3 culture at 60% confluency, with selection of transfectants in G418-supplemented growth medium, followed by isolation of 6-10 clones from each transfection and selection of individual transfectants again in Dulbecco's modified Eagle's minimal essential medium supplemented with G418 and 10% fetal calf serum (FCS), which were allowed to grow to confluence.

Second, with respect to the Examiner's point c) regarding Sugihara, Applicants respectfully submit that **Sugihara describes a cell mitogenic assay using incorporation of ^{3}H-thymidine during the cell cycle as a means of measuring cell proliferation**. In contrast, **the present invention provides a method for testing the survival of cells**, that is, their ability to overcome cell death, **as measured by a cell viability assay**. These **traits are quite different**, because viable cells are not necessarily undergoing mitosis. As a result, Applicants respectfully submit that Sugihara teaches that the level of cell proliferation, not the level of cell survival, of HUVEC cells treated with conditioned media from either VEGF115 or VEGF164 is significantly higher than that of the control cells. The Examiner cites Figure 5B in Sugihara as evidence of cell survival, but Applicants respectfully note that this figure shows the ^{3}H-thymidine labeling index of various experimental and control populations, including VEGF115 and VEGF164, as **evidence for cell proliferation**. Applicants agree with the Examiner that the **Sugihara does not teach measuring cell**

survival by tetrazolium (MTS)/formazan assay, but Applicants respectfully submit that **Sugihara does not teach measuring of cell survival at all.**

With respect to Buttke, Applicants agree that Buttke teaches an MTS/formazan assay (see page 12 of the specification). Applicants submit, however, that **Buttke repeatedly distinguishes between the use of the ^{3}H-thymidine assay to measure cell proliferation and the use of the MTS/formazan assay to measure cell viability**. Moreover, Buttke repeatedly emphasizes **the use of both assays to distinguish between cell proliferation and cell viability**, such as those described on page 238 with the FDC-P1 cell line.

In part, in the section entitled "Use of MTS/PMS and [^{3}H]TdR to distinguish cell viability from proliferation," Buttke states:

> Lastly, studies were done with the FDC-P1 cell line which has **an absolute requirement for IL-3 to traverse the cell cycle** (London et al., 1987). In contrast to many other lymphokine-dependent cell lines, such as HT-2 and CTLL-2, **depriving FDC-P1 of IL-3 does not result in an immediate loss of cell viability**. Rather, FDC-P1 cells deprived of IL-3 complete ongoing rounds of cell division, but are subsequently arrested in the G_0 stage of the cell cycle, wherein they can remain viable for 24-48 h (London et al., 1987). Thus, for at least a short time following IL-3 deprivation, **cell viability is maintained in the absence of proliferation**. It was therefore **of particular interest to compare MTS-formazan production with [^{3}H]TdR uptake** in FDC-P1 cells. (Buttke et al., p. 238; emphasis added.)

After describing the techniques used, Buttke outlines the results:

> During the initial 4 h period following IL-3 addition, FDC-P1 cells showed virtually no production of MTS-formazan and no incoporation of [^{3}H]TdR, consistent with their being arrested in G_0, and hence metabolically quiescent. By 24 h after IL-3 addition, FDC-P1 cells showed maximum incorporation of [^{3}H] TdR, implying that most of the cells had entered the 'S' phase of the cell cycle. During the same time interval (0-24 h) there was also an increase in MTS reduction. **However, while [^{3}H]TdR uptake subsequently declined, production of MTS-formazan did not peak until 1-2 days later.** As was initially pointed out byo Mosmann (1983), **the color change resulting from**

> **tetrazolium dye reduction should be <u>more indicative of the number of viable cells rather than proliferation</u>** per se. Hence, the results in Fig. 6 are most consistent with increased DNA synthesis during the first 24 h after IL-3 addition, followed 24-48 h later by an actual increase in cell number. (Buttke et al., p. 238; all emphasis added.)

Because **the cell proliferation assay of Sugihara and the cell viability assay of Buttke are measuring two different parameters, Buttke cannot supply the deficiencies of Sugihara**. There is **no motivation** for one of skill in the art **to use the stable transfection technique and cell proliferation assay** of Sugihara **to perform the transient transfection and cell viability assay** of the present invention. The cell proliferation assay described in Sugihara does not necessarily provide a measurement of cell viability as provided according to the present invention or as provided in Buttke. Moreover, there **is no suggestion in Sugihara** that measurement of cell viability, as opposed to cell proliferation, would be desirable.

Likewise, Buttke not only fails to remedy the deficiencies of Sugihara, but there is **no suggestion in Buttke that measurement of cell proliferation would necessarily be interchangeable with measurement of cell viability**. While Buttke provides instances in which cell proliferation and viability coincide, Buttke also provides **an example**, described above, in which **cell proliferation ceases while cell viability continues**.

In essence, therefore, **Buttke teaches away from Sugihara** by emphasizing a marked preference for **the need for both tests** as a means of **measuring and comparing two different cell parameters**, including an example in which **<u>the results of the two assays differed precisely because they were measuring two different parameters</u>**. As a result, **any technical advantages** of the MTS/formazan assay over the 3H-thymidine assay are **essentially irrelevant**, because the assays are **measuring two different cell parameters**.

Finally, there is **no discussion, either in Sugihara or in Buttke, of testing a plasmid for use in human gene therapy treatment**, as provided in **claims 9 and 17**.

Applicants respectfully submit that the present claims 1-3, 7-12, 16, and 17 fulfill the requirements of 35 U.S.C.§103(a) and request the Examiner's reconsideration of these claims accordingly.

VI. Rejection of Claims 6 and 15 under 35 U.S.C. §103(a) over Sugihara, in view of Buttke and Delli-Bovi, is Traversed

The Examiner has rejected claims 6 and 15 under 35 U.S.C. §103(a) "as being unpatentable over Sugihara et al., in view of Buttke et al. and Delli-Bovi et al." We disagree.

The Examiner alleges:

> The teachings of Sugihara et al and Buttke et al. are discussed above. However, **Sugihara et al. do not teach using Cos-1 cell line as host cells expressing the endothelial mitogen protein**.
>
> Delli-Bovi et al. teach a method of testing whether the K-FGF protein would promote the NIH3T3 cells in conditioned medium from host cells transfected with vector expressing K-FGF (see page 2938, 1st paragraph, lines 1-7). Delli-Bovi et al. teach that the conditioned medium collected from Cos-1 cells transfected with plasmid expressing K-FGF and added to the growth medium ofNIH3T3, and **the cell number is measured subsequently** (see Figure 6).
>
> It would have been obvious to one of ordinary skill of art to used the method taught by Sugihara et al. and using Cos-1 cell as host cell for expressing endothelial cell mitogen protein because both NIH3T3 cells and Cos-1 cells are widely used in transfection experiment and expressing a protein of interest. The ordinary artisan would have been motivated to use either Cos-1 or NIH3T3 as host cells because **both Sugihara et al. and Delli-Bovi et al. teach that conditioned media from either cell line transfected with endothelial mitogen**

protein promotes endothelial cell growth. The level of skill in the art is high. Absent evidence to the contrary, one of ordinary skill in the art would have reasonable expectation of success to use Cos-1 cells to express a biological active endothelial mitogen protein. Therefore, the invention would have been *prima facie* obvious to one of ordinary skill of art at the time the invention was made. (Pp. 5-6.)

Applicants respectfully disagree with the Examiner's comments and traverse the obviousness rejection.

Sugihara and Buttke have been discussed at length, *supra*. The same arguments outlined by Applicants in the previous section also apply here.

Delli-Bovi describes a method in which COS-1 cells, a standard cell line, are used as the transfection host cell line for an assay measuring plasminogen activator (PA) activity (see Delli-Bovi et al., pp. 2933-2934). In the section entitled "Biological activity" (p. 2936), Delli-Bovi states:

> We previously reported that K-FGF was mitogenic for BHK21 and NIH 3T3 cells made quiescent by serum starvation (4). Since FGFs are thought to be important angiogenic factors and to stimulate the proliferation of and protease production by endothelial cells in culture [citations omitted], we tested the effect of our protein on BCE cells. **We measured the induction of PA activity, DNA synthesis, and cell proliferation**, and the results were compared with those obtained with bFGF. Conditioned medium produced by COS-1 cells transfected with the p9BKS3A plasmid, which contains the K-*fgf* cDNA, was used as a source of crude K-FGF…This medium effectively stimulated PA production in BCE cells when the medium was assayed in the presence of heparin (Fig. 4)….The culture medium from transfected COS-1 cells also **stimulated DNA synthesis** in growth-arrested BCE cells (Fig. 5). This medium, in the presence of heparin, was almost as stimulatory as the medium supplemented with bFGF, whereas the medium from control COS-1 cells was nonstiulatory. **A proliferative effect** of the K-FGF was also apparent under these conditions when the number of cells was determined (Table 1). (P. 2936; italics in original; bold emphasis added.)

A review of Figure 5 (p. 2937) and its accompanying legend outlines the method and results of ^{3}H-thymidine incorporation assays as a means of measuring DNA synthesis. Table 1 (p. 2938) and Figure 6 (p. 2938) show the "proliferative effect." Delli-Bovi states that what was measured was the induction of **PA activity, DNA synthesis, and cell proliferation – <u>not cell viability</u>** as provided by the present invention. The only mention of cell viability is "lost viability" (p. 2938), which occurs when cells not only fail to proliferate, but **decrease** in number.

The teachings of Delli-Bovi fail to supply the deficiencies of Sugihara and Buttke. Delli-Bovi does not describe a cell viability assay, but rather describes the type of ^{3}H-thymidine incorporation assay used by Sugihara and Buttke to measure cell proliferation, along with a cell counting assay also used to measure cell proliferation. While Delli-Bovi describes a decrease in cell number as "lost viability," an increase in cell number clearly measures proliferation and clearly this parameter, in light of the DNA synthesis experiments, is the important parameter for Delli-Bovi. A lack of change cannot automatically be equated with viability, because it might simply result from an equilibrium between cell proliferation and cell death. Clearly, a mere cell count cannot distinguish between the two parameters of cell viability and cell proliferation.

Moreover, there is no suggestion in Delli-Bovi to combine the teachings of Delli-Bovi with those of Sugihara and/or Buttke. Like Sugihara, Delli-Bovi measures DNA synthesis and cell proliferation – not cell viability. Delli-Bovi does not distinguish between the two parameters. As a result, the use of COS-1 cells in the Delli-Bovi assay is irrelevant, because the parameters being measured are different.

Applicants respectfully submit that the present claims 6 and 15 fulfill the requirements of 35 U.S.C.§103(a) and request the Examiner's reconsideration of these claims accordingly.

VII. Conclusion

In view of the foregoing amendments and remarks, the present application is respectfully considered in condition for allowance. An early reconsideration and notice of allowance are earnestly solicited.

It is believed that all outstanding rejections have been addressed by this submission and that all the claims are in condition for allowance. If discussion of any amendment or remark made herein would advance this important case to allowance, the Examiner is invited to call the undersigned as soon as convenient.

Applicants hereby request a two-month extension of time for the Amendment and accompanying materials and hereby submit the requisite fee accordingly. If a petition for an additional extension of time is required, then the Examiner is requested to treat this as a conditional petition for an additional extension of time. Although it is not believed that any additional fee (in addition to the fee concurrently submitted) is required to consider this submission, the Commissioner is hereby authorized to charge our deposit account no. 04-1105 should any fee be deemed necessary.

Respectfully submitted,

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Kathryn A. Piffat, Ph.D.

Kathryn A. Piffat, Ph.D. (Reg. No. 34,901)
Intellectual Property Practice Group
EDWARDS & ANGELL, LLP
P.O. Box 9169
Boston, Massachusetts 02209
Telephone: 617-439-4444

Customer No. 21874